CAPSULES notes and applications from BASi

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373

Paclitaxel

LC/MS/MS Determination of Paclitaxel and Metabolites in Human Plasma Using An Isotopically-Labeled Internal Standard

Method Profile

Liquid-liquid extraction for fewer interferences
Less than 0.5 mL plasma required
Rapid sample turnaround
Linear over wide range
LC/MS/MS for maximum specificity
Unparalleled precision and accuracy through use of a stable-label internal standard

Sensitivity

Paclitaxel LOQ: 0.1 ng/mL
6α-hydroxy paclitaxel LOQ: 0.1 ng/mL
3'-p-hydroxy paclitaxel LOQ: 0.1 ng/mL

BASi CRS has incorporated a unique LC/MS/MS method for simultaneous quantitation of paclitaxel and two metabolites (6α-hydroxy paclitaxel and 3'-*p*-hydroxy paclitaxel) in human and dog plasma. The method has been rigorously validated under GLP over a range of 0.1-100 ng/mL. The method requires less than 0.5 mL of plasma, is highly selective for the analytes, and can be extended to other metabolites of paclitaxel. This is the *only* paclitaxel assay available that uses a $^{13}C_6$-isotopically labeled internal standard.

Paclitaxel, originally isolated from the bark of the Pacific Yew tree, is used in cancer therapy. Other therapeutic applications of paclitaxel are being developed. Lower limit of quantitation (LOQ) of the method allows determination of subnanogram/mL levels for pharmacokinetic studies, as well as for testing the effects of co-administered drugs.

The present method makes optimum use of a LC separation. A liquid-liquid extraction cleanup step provides cleaner samples, resulting in longer column lifetimes and fewer potential interferences injected into the LC/MS. By using carefully optimized LC conditions, a robust separation is obtained, enabling the mass spectrometer to provide maximum sensitivity while minimizing the chances of signal loss due to coeluting interferences. The method's 4.5-min cycle time allows analysis of a 250-sample run in one overnight sequence which results in higher quality data with shorter turn around times and fewer repeated samples due to rejected chromatograms and failing runs.

Method Performance Data

A ten-point standard curve (SC) was run during the validation. The data indicate excellent linearity, precision, and recovery as shown for the sampling of data in **T1** and **T2**.

Quality Control (QC) samples are plasma samples spiked with the analytes at levels simulating those expected in real samples. Four QC levels (one low-level, two mid-range, and one high-level) were injected (n = 6) in each of three runs. Excellent accuracy and precision were observed for these samples in three consecutive runs as shown in **T3**. Recovery was greater than 90% for all samples tested.

The paclitaxel method was subjected to normal sample handling challenges, including stability in plasma, stability of extracted samples, the effects of freeze-thaw, and dilution of over-curve samples. In all cases, the method maintained the requisite precision and accuracy for paclitaxel as well as the two hydroxy-metabolites.

The true performance of an analytical method is its ability to provide reliable results on a continuous basis. BASi has analyzed more than 10,000 plasma samples for paclitaxel, proving the ruggedness of LC/MS/MS methodology for this task.

T1. Between-run (n = 3) accuracy and precision for injections of extracted standards.

	Paclitaxel		6α-hydroxy paclitaxel		3'-*p*-hydroxy paclitaxel	
Sample name	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**
SC-0.1 ng/mL	100%	1.1%	99%	1.7%	99%	0.9%
SC-0.3 ng/mL	101%	3.4%	105%	3.4%	104%	2.4%
SC-1 ng/mL	96%	2.1%	98%	4.4%	98%	2.5%
SC-10 ng/mL	101%	2.0%	99%	3.7%	99%	3.1%
SC-100 ng/mL	106%	4.1%	104%	3.1%	102%	2.2%

T2. Typical linearity data for standard curve (0.1 to 100 ng/mL).

Sample name	**r^2**
paclitaxel	0.9996
6α-hydroxy	0.9994
3'-*p*-hydroxy	0.9994

T3. Between-run (n = 18) accuracy and precision for injections of extracted spiked samples (QC samples).

	Paclitaxel		6α-hydroxy paclitaxel		3'-*p*-hydroxy paclitaxel	
Sample name	**Mean**	**CV**	**Mean**	**CV**	**Mean**	**CV**
QC-0.1 ng/mL	104%	8.4%	104%	6.0%	101%	7.3%
QC-0.3 ng/mL	103%	6.0%	101%	5.1%	100%	4.6%
QC-30 ng/mL	105%	4.2%	101%	4.7%	100%	4.8%
QC-100 ng/mL	99%	3.7%	95%	2.6%	95%	3.5%

Mass chromatograms of paclitaxel and its two hydroxy-metabolites. Complete resolution of all analytes is obtained in less than four min. Each analyte is determined by LC/MS/MS. In the first stage of the quadrupole, the mass spectrometer isolates the molecular ions as shown in the mass chromatograms. Then the added specificity of MS/MS detection is used to identify and quantify the analytes by their unique product ions. Thus specificity and low limits of detection are obtained with a high degree of accuracy and precision. A unique $^{13}C_6$- paclitaxel internal standard (patent applied for) adds method ruggedness by correcting for normal variations that happen during sample processing.

The chromatograms show that a significant signal is obtained at the lower end of the standard curve (0.1 - 0.3 ng/mL), as well as in the normal therapeutic range (100 ng/mL). The blank baseline shows that the method is interference-free, improving the precision and accuracy of the method as well as user confidence in the high quality of the results.